

86 63. (Amended) The sequence of claim 62, wherein said open reading frame is
87 open reading frame 17 (SEQ ID NO. 4), 19 (SEQ ID NO. 5), 43 (SEQ ID NO. 6), 102 (SEQ ID
88 NO. 7), 104 (SEQ ID NO. 8), or 182 (SEQ ID NO. 9).
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REMARKS

Applicant respectfully requests entry of the amendments shown above correlating SEQ ID Nos. with sequences in the description, claims and figures. No new matter is presented with these amendments.

Applicant believe that the NOTICE TO COMPLY was in error in indicating that an initial or substitute computer readable form and paper copy of the Sequence Listing, along with the required statement stating that the paper and computer readable forms are the same, as a corrected Sequence Listing was submitted 25 August 2000 and no defects were indicated with that submission. Therefore, only the specification, claims and drawings were amended to enter reference to appropriate SEQ ID Nos. If Applicant is in error, and a substitute Sequence Listing is required, the Examiner is respectfully requested to indicate what defects require correction.

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Applicant requests a two-month extension of time to allow timely filing of this response up to and including 12 July 2001. A check for that extension is enclosed herewith. If any additional fee is required in connection with the response, kindly charge the appropriate amount to Deposit Account 50-0872.

Respectfully submitted,

Date 12 Jul 2001

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APPENDIX 2: Amended Specification Paragraphs – Marked-up Copies

Page 46, paragraph beginning at line 12.

The shuttle vector pT0021, in which the firefly luciferase (*lucFF*) expression is controlled by the *ars* (arsenite) promoter/operator (Tauriainen et al., 1997), was modified in the following fashion. Two oligonucleotides corresponding to a short antigenic peptide derived from the hemagglutinin protein of influenza virus (HA epitope tag) were synthesized (Field et al., 1988). The sense strand HA tag sequence (with *Bam*HI, *Sal*I and *Hind*III cloning sites) is:

5'-gatcccggtcgaccaagctTACCCATACGACGTCCCAGACTACGCCAGCTGA-3' (SEQ ID NO. 11) (where upper case letters denote the nucleotide sequence of the HA tag); the antisense strand HA tag sequence (with a *Hind*III cloning site) is:

5'-agctTCAGCTGGCGTAGTCTGGGACGTCGTATGGGTAAagcttggtcgaccgg-3' (SEQ ID NO. 12) (where upper case letters denote the sequence of the HA tag). The two HA tag oligonucleotides were annealed and ligated into pT0021 vector which had been digested with *Bam*HI and *Hind*III. This manipulation resulted in replacement of the *lucFF* gene by the HA tag. This modified shuttle vector containing the *arsenite* inducible promoter, the *arsR* gene, and HA tag was named pTHA. A diagram outlining our modification of pT0021 to generate pTHA is shown in Fig. 1.

Page 46, paragraph beginning at line 28.

Each ORF, encoded by Bacteriophage 77, larger than 33 amino acids and having a Shine-Dalgarno sequence upstream of the initiation codon was selected for functional analysis for bacterial inhibition. In total, 98 ORFs were selected and screened as detailed below. A list of these is presented in Table 5. Each individual ORF, from initiation codon to last codon (excluding the stop codon), was amplified from phage genomic DNA using the polymerase chain reaction (PCR). For PCR amplification of ORFs, each sense strand primer targets the initiation codon and is preceded by a *Bam*HI restriction site (5'-cgggatcc³) and each antisense oligonucleotide targets the pentultimate codon (the one before the stop codon) of the ORF and is

preceded by a *Sal* I restriction site (5'gcgtcgaccg3') (SEQ ID NO. 1). The PCR product of each ORF was gel purified and digested with *Bam*HI and *Sa*II. The digested PCR product was then gel purified using the Qiagen kit as described, ligated into *Bam*HI and *Sa*II digested pTHA vector, and used to transform *E. coli* bacterial strain DH10 β (as described above). As a result of this manipulation, the HA tag is set inframe with the ORF and is positioned at the carboxy terminus of each ORF (pTHA/ORF clones). Recombinant pTHA/ORF clones were picked and their insert sizes were confirmed by PCR analysis using primers flanking the cloning site. The names and sequences of the primers that were used for the PCR amplification were: HAF:

5'TATTATCCAAACTTGAACA' (SEQ ID NO. 2); HAR:

5'CGGTGGTATATCCAGTGATT' (SEQ ID NO. 3). The sequence integrity of cloned ORFs was verified directly by DNA sequencing using primers HAF and HAR. In cases where verification of ORF sequence could not be achieved by one pass with the sequencing primers, additional internal primers were selected and used for sequencing.

Fig. 1A

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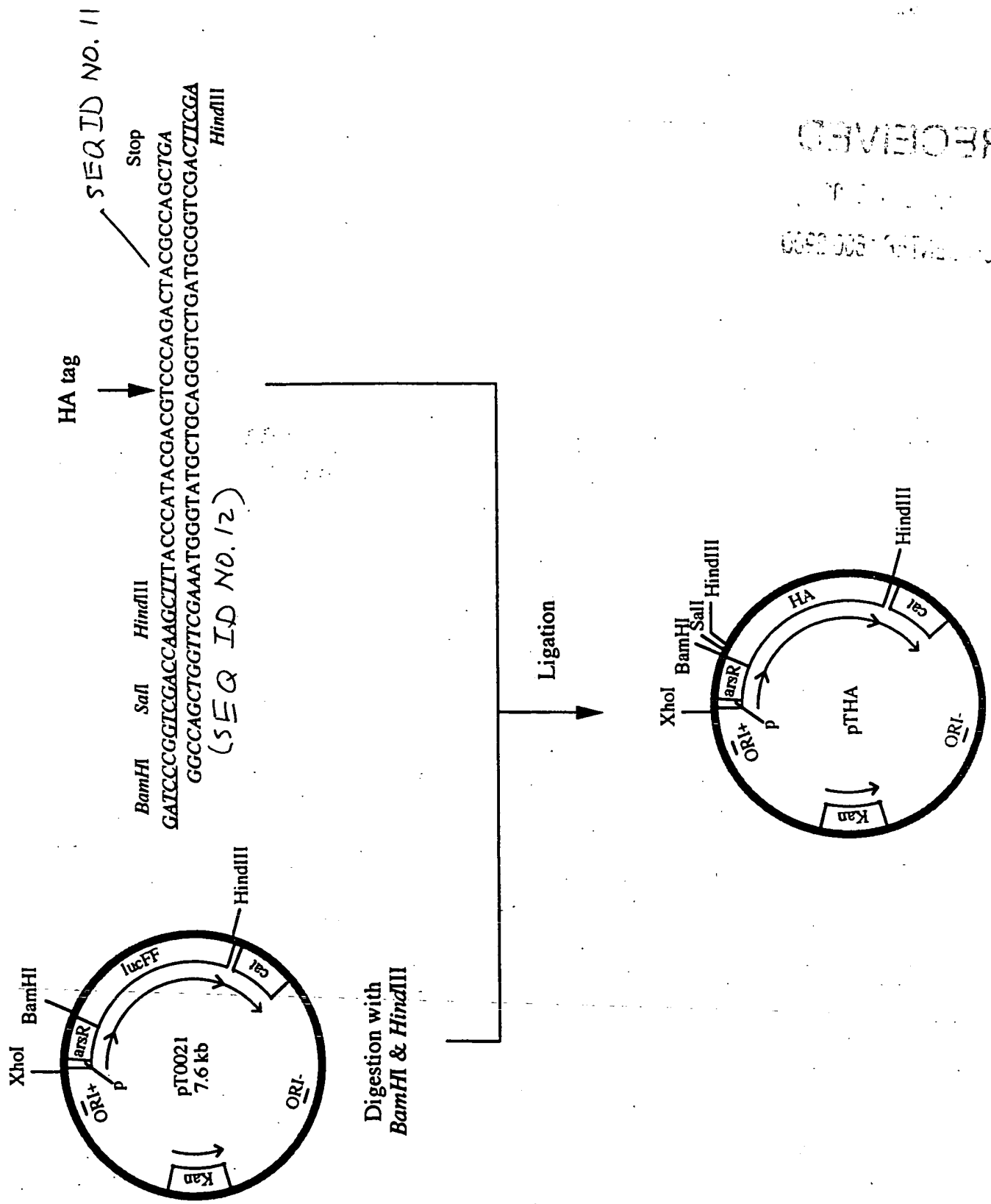
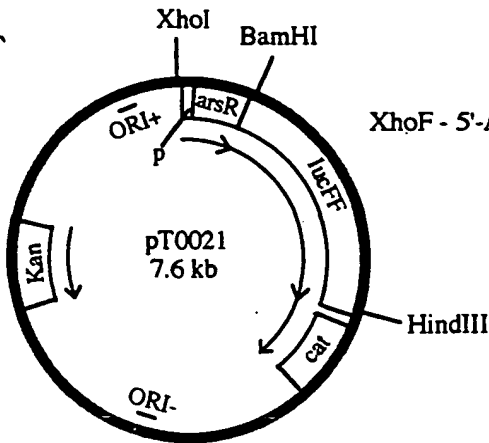


Fig. 1B

PCR of pT0021 with XhoF & BamHNR



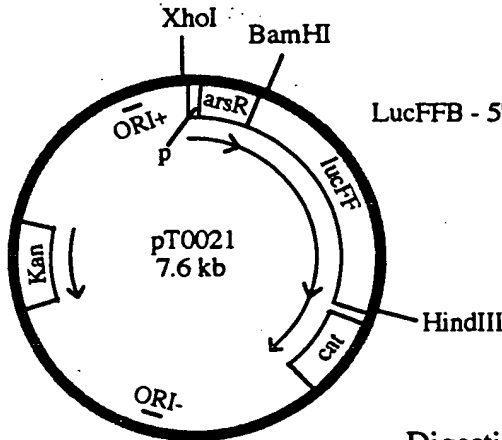
XhoF - 5'-AATT CTCGAGTAAAATAACAT-3' (SEQ ID NO. 13)
XhoI

(SEQ ID NO. 14)
AAATCAGGTGACTGT TGAGAAAAGGAGGCGGATCCCG BamHNR
Stop of *arsR* RBS BamHI

Digestion with
XhoI & BamHI

Ligation

Modified between stop
of *arsR* to *BamHI*



PCR of pT0021 with LucFFB & LucFFH

(SEQ ID NO. 15)
LucFFB - 5'- CGGGATCCATGAGGGGTTCGAAGACG
BamHI Start of LucFF Original BamHI was modified

(SEQ ID NO. 16)
GAAAGTCCAAATTGTAAGCTTGGG - LucFFH
Stop of HindIII LucFF

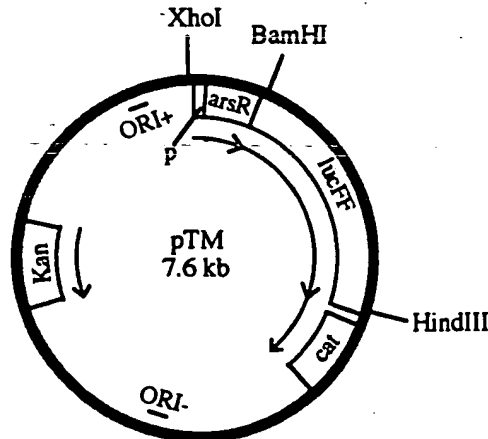
Digestion with
BamHI & HindIII

Ligation

Modified in the
vicinity of *BamHI*

Cloning site for ORFs:
BamHI & *HindIII*

No additional codons
in the induced protein



CTCGAG P ATG *arsR* TGA GAAAAGGAGGCGGATCC ATG LucFF TAA GCTT
XhoI RBS BamHI HindIII

(SEQ ID NO. 17)